



USDA ARS National Animal Germplasm Program

Boar Semen Collection and Dilution Protocol

Semen collection:

Prepare the semen extender (e.g. for Androhep Plus or Androstar mix 1 packet of powdered medium with 1 L of distilled/deionized water, Minitube, Verona, WI) and warm to 37 °C.

Collect the sperm-rich fraction of a boar semen sample using the hand-glove technique or a phantom mount and remove the gel fraction with sterile gauze or a semen filter.

Determine the sperm-rich ejaculate volume, sperm concentration, and the volume of the ejaculate that is needed:

1. sperm-rich ejaculate volume X sperm concentration = sperm count;
2. sperm count ÷ 45 billion sperm = sample volume needed by USDA.

NOTE: To meet repository targets, 45 billion cells are needed per boar collection for commercial boars and the entire ejaculate for minor/heritage breed boars; this will be determined by NAGP staff and communicated to the cooperator prior to the collection of the samples. Once determined, dilute the sample as follows:

Dilute the required sperm-rich fraction 1:1 (v:v) with 37 °C semen extender in a 37 °C beaker.

Aliquot the sample into 50 mL centrifuge tubes that are labeled with the name and identification number of the boar.

Cool the sample to 23 °C over 1 hour by placing it on the laboratory bench. The sample must be shielded from light during this time.

Further cool the sample over 1.5 hours by placing it in a 15 °C refrigerator and maintain it at this temperature prior to packaging for delivery. The samples are then at the appropriate temperature for overnight transportation to the repository.

Transportation:

Please see the Impact Shipper Protocol on the Animal GRIN webpage noting the specific temperatures for each species and type of tissue:

<https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/animal/animal-protocols/>

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